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Assessment of anti bacterial screening of *Pongamia pinnata* stem against bacterial species: An *In-vitro* approach

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ABSTRACT

Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non-nutrient molecules which protect the human body against various pathogens. Nature has been a source of medicinal agents for thousands of years and a large number of modern drugs have been isolated from natural sources. Herbal medicine is the oldest known healthcare system known to mankind. India has rich medicinal plants of nearly 7500 species. Many medicinal plants were with a long history of use in folk medicine against a variety of diseases. Recently, many researchers have taken a great interest on medicinal plants for their phytochemical constituents and biological activities including anti microbial activity. The anti bacterial activity of the ethanolic crude stem extract of *Pongamia pinnata* against four bacterial species (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*) was investigated, using agar diffusion technique. At concentrations ranging from 10-40 mg/mL, the ethanolic crude extract showed activity against the four bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *E. coli*) from 2 mm-20 mm, after 24 hours incubation. The present study showed the effectiveness of the crude plant extract against the tested bacterial strains and indicates the potential use of the extract as anti bacterial agent for the control of infectious diseases.

Keywords: *Pongamia pinnata*, anti bacterial activity, agar diffusion technique, bacterial strains, chloramphenicol.

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INTRODUCTION

Finding the healing power in plants dates back to Vedic times. Having the divine origin Ayurveda, the science of life relies heavily on the plants for the therapeutic uses. There is a constant search for new compounds which have health benefits and in this regard the medicinal plants offer wide opportunities for scientific research as they are rich in phytochemicals such as flavonoids, lignin, phenolic acids and tannins. Priorities are given on the anti-microbial, anti-mutagenic and anti-carcinogenic activity of the phytochemical compounds[1].

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality especially in immune-compromised patients in developing countries[2]. The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds the urgency to the search for new infection-fighting strategies[3,4]. For a long time, plants have been an important source of natural products for human health. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties[5]. Plants have many anti-microbial properties as secondary metabolites such as alkaloids, phenolic compounds, etc. The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections[6,7].

The role of traditional medicines in the solution of health problems is invaluable on a global level. Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine[8]. With the associated side effects of the modern medicine, traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions[9]. Research work on medicinal plants has intensified and information on these plants has been exchanged. This research will go a long way in the scientific exploration of medicinal plants for the

benefit of man and is likely to decrease the dependence on synthetic drugs[10].

Pongamia pinnata (Linn.) Pierre is a medium sized glabrous tree popularly known as Karanja in Hindi, Indian Beech in English, Kanuga in Telugu, Naktamala in Sanskrit, Sukh Chain in Urdu and Pongam in Tamil[11]. Most of the Tamil Nadu physicians of Indian system of traditional medicine Ayurveda and Siddha use *Pongamia pinnata* to treat various kinds of diseases including diabetes mellitus[12]. It is a medicinal plant native to Western Ghats and chiefly found in tidal forests of India[11]. *Pongamia pinnata* also called as *Derris indica*, is a monotypic genus and grows abundantly along the coasts and riverbanks in Myanmar. The tree is known for its multipurpose benefits and as a potential source of biodiesel[13]. The seeds are reported to contain on average about 28-34% oil with high percentage of polyunsaturated fatty acids[14]. Historically, *Pongamia* has been used as folk medicinal plant, particularly in Ayurvedha and Siddha systems of Indian medicine[15]. All parts of the plant have been used as a crude drug for the treatment of tumours, piles, skin diseases, itches, abscess, painful rheumatic joints wounds, ulcers, diarrhea etc[16]. Besides, it is well known for its application as animal fodder, green manure, timber and fish poison. It has also been recognized to possess applications in agriculture and environmental management, with insecticidal and nematicidal activity. More recently, the effectiveness of *P. pinnata* as a source of biomedicines has been reported[17], specifically as antimicrobial and therapeutic agents. This study therefore was an attempt to determine the anti bacterial potentials of the ethanolic stem extract against four bacterial species namely *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.

Taxonomy[18]

Kingdom – Plantae

Subkingdom – Tracheobionta

Superdivision – Spermatophyta

Division – Magnoliophyta

Class – Magnoliopsida

Subclass – Rosidae

Order - Fabales

Family - Fabaceae

Genus - *Millettia*

Species - *Millettia pinnata* (L.) Panigrahi

Figure 1: *Pongamia pinnata* plant

Figure 2: *Pongamia pinnata* stem

Geographical distribution:

It is widely distributed throughout tropical Asia and the Seychelles Islands, South Eastern Asia, Australia, India and locally distributed throughout the State of Maharashtra (India) along the banks of rivers; very common near the sea-coast in tidal and beach-forests in Konkan; along Deccan rivers[19].

MATERIALS AND METHODS

Collection of plant material

The plant material was collected from surrounding areas in Hyderabad, Telangana, India. The fresh stem was collected and washed with tap water and latter with demonized water and dried under shade. The plant material was regularly checked for fungal growth or rotting. After the plant material was dried it was powdered with the help of an electric blender and sieved through size 80 sieve to obtain a uniform fine particle size. This plant material was stored in airtight containers at 4 ^{0}C.

Preparation of plant extract

50 gms of the sieved powder was accurately weighed and extracted with solvent like ethanol in a Soxhlet extractor for 72 hours. The extract thus obtained was concentrated under reduced pressure to yield crude plant extract. These crude plant extract was reconstituted in Dimethyl sulphoxide (DMSO) maintaining the concentration of 10000 µg/ml. The extract was stored at 4 ^{0}C in amber coloured glass stoppered vials for further use.

Test cultures

The following strains were used for the study and were maintained by the Department of Bio-technology, School of Pharmacy, Anurag Group of Institutions, Venkatapur.

- ✓ *Escherichia coli*
- ✓ *Pseudomonas aeruginosa*
- ✓ *Staphylococcus aureus*
- ✓ *Bacillus subtilis*

These cultures were maintained on sterile nutrient agar slants and stored at 4 ^{0}C until further use.

***In-vitro* anti-bacterial activity screening**

The anti bacterial activity of the crude extracts was determined in accordance with the agar well diffusion method. The bacterial isolates were first grown in a nutrient broth for 6 h and standardized to 0.5 McFarland standard before inoculation on to the media. Wells were then bored onto the agar using a sterile 4 mm diameter cork borer. The crude extracts at 1-4 mg/mL were introduced into the wells for about 30 min. The plates were incubated for 24 hours. Observation for zones of inhibition after 24 hours was done. The activities of the crude extracts were compared to that of chloramphenicol (control) at a concentration of 1-4 mg/mL, respectively, and the results were recorded[20,21].

RESULTS AND DISCUSSION

The ethanolic stem extract of *Pongamia pinnata* was selected to screen for its anti bacterial activity against different bacterial strains. Table 2 shows the susceptibility pattern of the organisms to the crude extract of *Pongamia pinnata*. The ethanolic extract had effect on all the organisms at concentration from 10-40 mg/mL and the zone of inhibition ranged from 2-20 mm in diameter. The control recorded zone of inhibition ranging 1-18 mm.

Table 1: Anti bacterial activity of bark of *Pongamia pinnata* and the control drug after 24 hours of incubation

Test organism	Concentration of the crude extract (mg/mL)	Zone of inhibition for crude extracts (mm)	Zone of inhibition for control drug (mm)
Escherichia coli	40	12	8
	30	10	5
	20	7	2
	10	3	1
Pseudomonas aeruginosa	40	9	-
	30	7	-
	20	4	-
	10	2	-
Staphylococcus aureus	40	20	18
	30	12	12
	20	8	8
	10	4	3
Bacillus subtilis	40	20	18
	30	18	6
	20	12	4
	10	5	2

' – ' indicates no activity

Plants are important source of pharmacophore which will function as new chemotherapeutic agents. The first step to develop a chemotherapeutic agent from plants would be the assay of in vitro anti bacterial activity. The extracts thus found active will help to identify the active compounds responsible for the activities from the plant. In recent years multi drug resistance is seen in pathogenic bacteria which has revived interest in the search of new antibacterial agents from natural sources. In fact, gram negative bacteria *P. aeruginosa* are frequently reported to have developed multi drug resistance to many of the antibiotics. But, the extract shows a good activity against bacterial strains. The antibacterial agents from natural sources also eliminate the side effects of synthetic or semi synthetic antibacterial agents. The anti bacterial activity of the plant extract was variable with various organisms. The zones of inhibitions ranged from 2 mm to 20 mm. In this study, the anti bacterial potentials of the ethanolic crude extract of the stem of *Pongamia pinnata* was investigated. The anti bacterial effect of the crude extract of the *Pongamia pinnata* was determined in comparison with the effect of chloramphenicol (control drug) against the test organisms. The crude extracts had more inhibitory effects compared to the antibiotic chloramphenicol (control drug) used. This could be attributed to the presence of phytochemical constituents found in the crude extract of the *Pongamia pinnata*. It indicates that the plant has effective phytochemical constituents against the pathogens which were highly active even in low concentration. The presence of bioactive compounds and supporting studies reveals that anti bacterial activity of *Pongamia pinnata* which can be used to discover bioactive natural products.

CONCLUSION

From the above study it is evident that the stem of *Pongamia pinnata* have significant anti bacterial action and can be employed as an anti bacterial agent. Also fractionation of the extract to yield anti bacterial compounds should be carried out. Further studies should be conducted on the acute and sub chronic toxicity of the crude extract. Similarly, determination and characterization of active compound(s) in the crude extracts should be conducted. The anti bacterial assessment of *Pongamia pinnata* against the selected pyogens reveals that the phytoconstituents has high anti bacterial property. Since the drug resistance nature of the pyogens increases day by day this herbal remedies will serve as an alternative medicine without side effects. These extract was used as medicine for pyogenic infection instead of using synthetic antibiotics and drugs. These plants can be used to discover bioactive natural products that serve as lead for the development of new phytopharmaceuticals.

In spite of the fact that these extract is not pure compound, the results obtained clearly suggest the potency of the extracts. Thus, the extract can further be fractionated to yield anti bacterial compounds which can be used in the development of phytomedicine against these microbes. Inhibitory activities of the crude extracts are in general greater or comparable to that of the standards used. These extracts being of natural origin are safer and less prone to

development of drug resistance in bacteria. The above mentioned pathogens cause a number of life threatening diseases which can be managed by the use of synthetic antibiotics which have their own side effects. The increasing problems associated with drug resistance in bacteria and the increasing cost of synthetic anti bacterial agents pharmaceutical companies are looking for other alternatives. Our aim is to find plants which have antibacterial activity without many side effects. A detailed study needs to be carried out to isolate bioactive compounds that show anti bacterial activity.

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AUTHOR'S CONTRIBUTION

All authors contributed to the design and implementation of the research.

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